

PHYSICAL DIFFERENCES AMONG CLASS II HYDROPHOBINS AFFECT THEIR SELF ASSEMBLY MECHANISM HENCE THEIR GUSHING POTENTIAL

Riveros Galan David^{1*}, Shokribousjein Zahra¹, Mandelings Nathalie¹, Gebruers Kurt¹, Michiels Christian¹, Delcour Jan¹, Derdelinckx Guy¹

¹ Department of Microbial and Molecular Systems (M²S), CLMT, Leuven Food Science and Nutrition Research Centre (LFoRC), Kasteelpark Arenberg 22, bus 2463, BE-3001 Heverlee, Belgium

*:Davidsantiago.riverosgalan@biw.kuleuven.be

Introduction: Hydrophobins and primary gushing

Class II Hydrophobins are fungal amphipilic surface active proteins, they are produced during their vegetative growth covering spores and hyphae to make them hydrophobic and more resilient to the weather conditions(Linder, 2005). Primary gushing is a physical phenomenon caused by the interaction of hydrophobins with gaseous CO₂ producing spontaneous overfoaming out of the container without any shaking. Through trapping CO₂ into nanobubbles structures (Fig. 1) (Deckers et al, 2012) stabilizing and solubilizing it, when the bottle is opened the sudden pressure drop will explode the nanobubbles realeasing all its energy causing gushing.

However , it has been observed that when varies the hydrophobin involved in primary gushing the amount of overfoaming is different (Sarlin, 2012). Although all class II hydrophobins share a similar globular shape with eight conserved cysteines within their sequences and four disulfide bridges, little is known about how minor differences in sequences, protein folding and protein-protein interactions have direct effect on their interaction with other hydrophobic surfaces and hydrophobic molecules like CO₂. This research tries to elucidate how this process is achieved and how this information can be used to understand deeply primary gushing.

Materials and methods



Gushing potential determination of four different class II hydrophobins: HFBI, HFBII (*Trichoderma reesei*), HFB2-a2 (*Trichoderma harzianum*) and Fghyd5 (*Fusarium graminearum*)

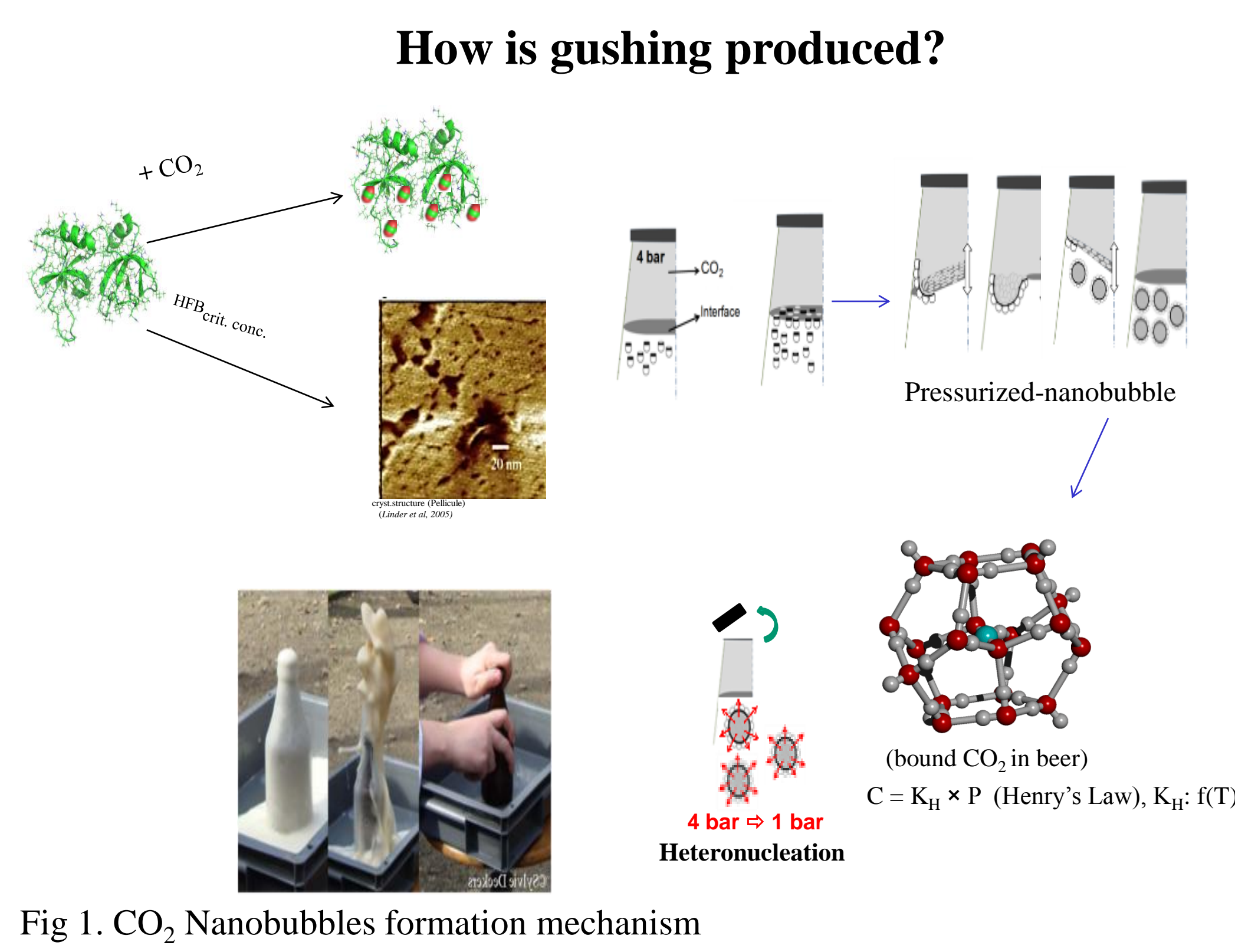
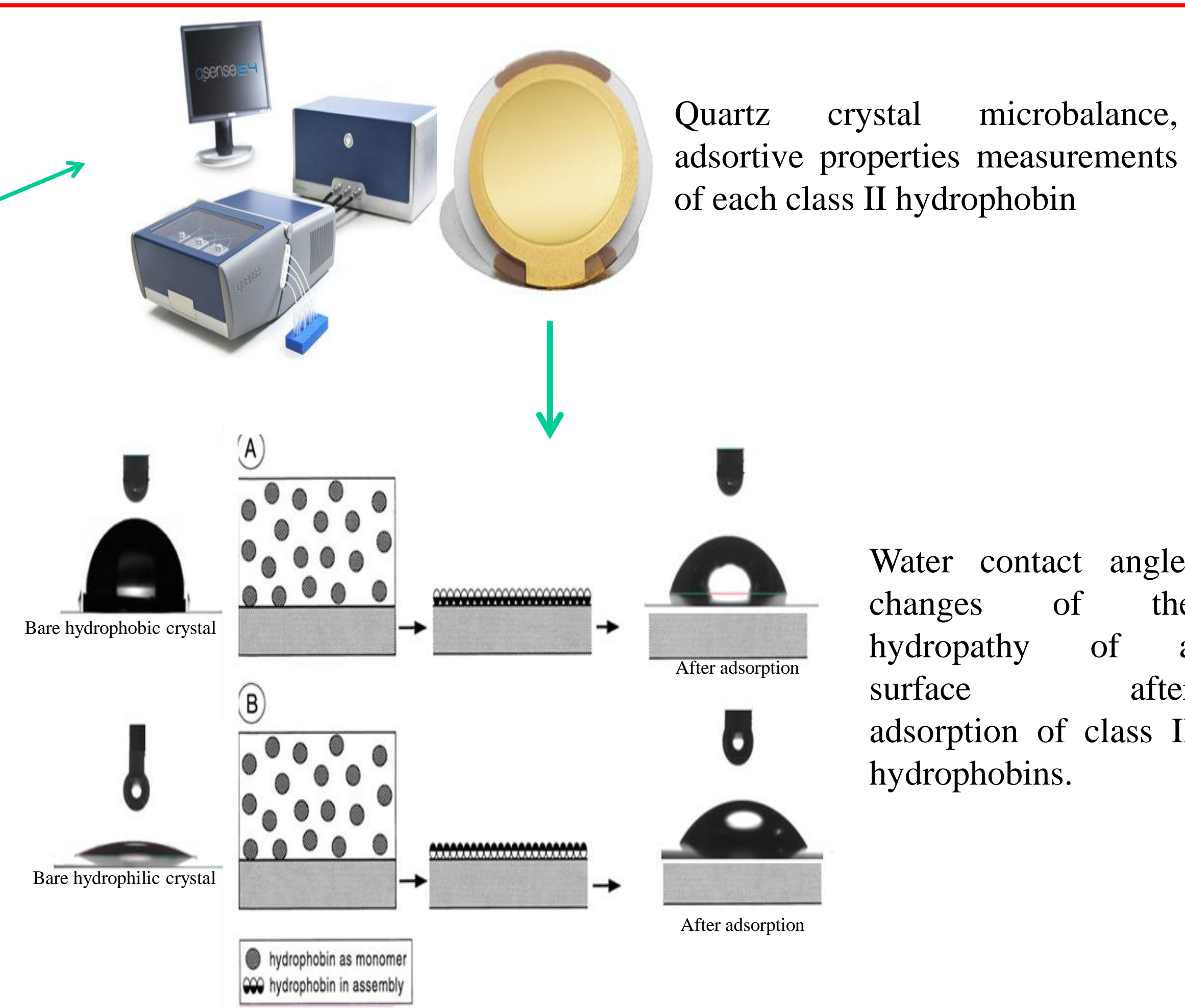


Fig 1. CO₂ Nanobubbles formation mechanism

Results and discussion

Hydrophobin amount added (ug/L)	Amount of sparkling water gushed (mL) (n=3)			
	HFBI	HFBII	Fghyd5	HFB2-a2
0,3	0	0	0	0
3	12	15	0	18
30	145	146	0	254
50	191	185	67	270
100	273	260	234	353
150	490	484	352	497
200	516	509	480	562
250	614	614	512	652
300	608	609	548	657

Table 1. Gushing potential determination

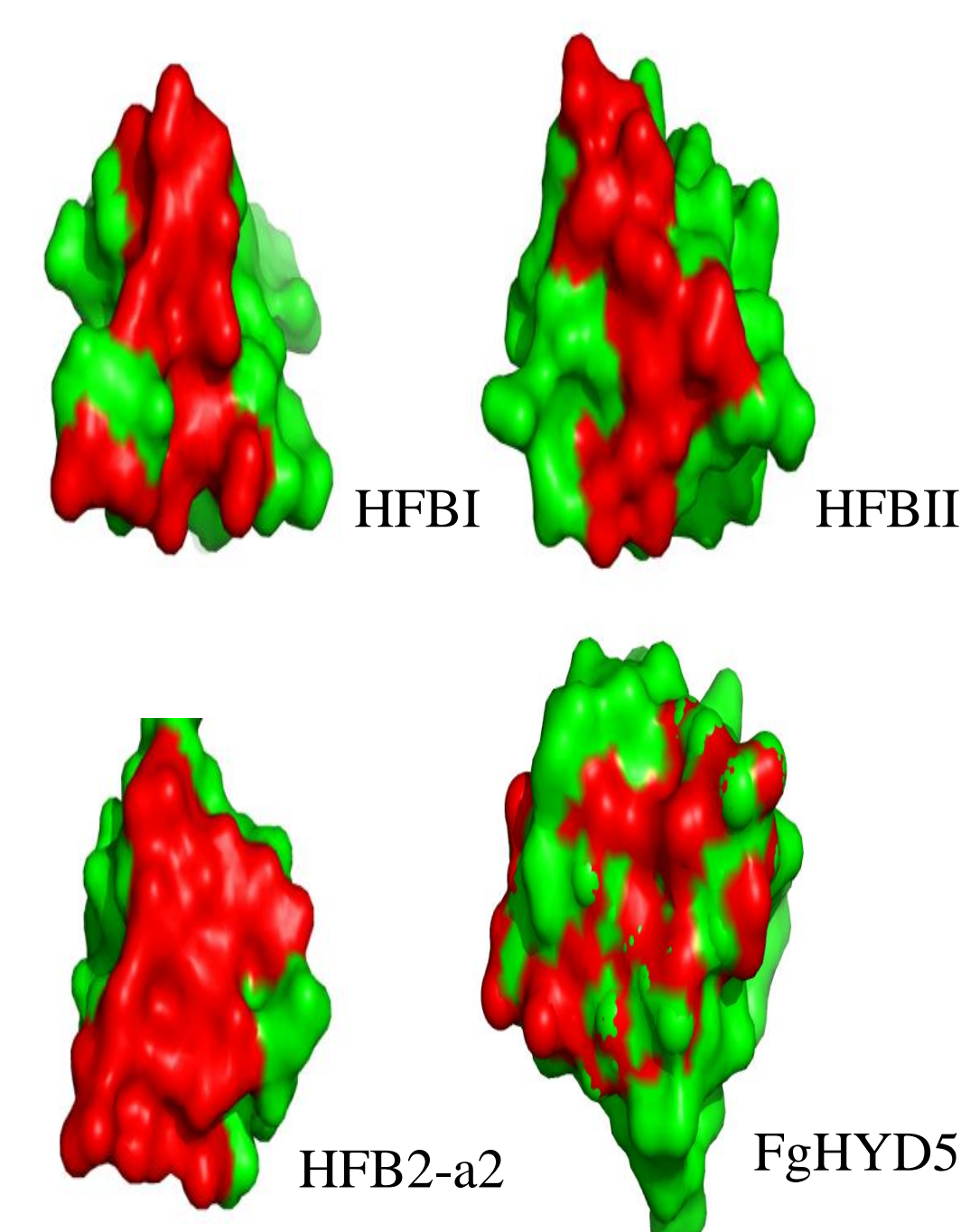


Fig 3. Molecular models of hydrophobin monomers, hydrophobic patches are colored in red

Gushing potential was evaluated with four different class II hydrophobins HFBI and HFBII from *Trichoderma reesei*, Fghyd5 from *Fusarium graminearum* and HFB2-a2 from *Trichoderma harzianum* (Table 1.). It was noticed that for Fghyd5 it was needed 10 times more hydrophobin to produce the same amount of overfoaming compared with the other three. On the other hand HFB2-a2 exhibited a stronger gushing potential, reaching high overfoaming at low concentrations (30ug/L). To elucidate the reason of this behavior QCM adsorption was used to understand the behavior of this proteins with different hydrophathys.

A detailed observation of the hydrophobic patches of the four tested hydrophobins showed that their size and uniformity change among them (Fig. 3). Biomolecular modelling was used using Rosetta server to understand the protein-protein interactions when a monolayer is formed. The results showed that in the case of HFBI and Fghyd5 a glutamine residue is protruding from the hydrophobic patch augmenting the distance between the hydrophobic surface or molecule (CO₂) making it weaker. On the other hand no glutamine residue was founded in HFB2-a2 (it was buried within the structure)(Fig. 4). This facts can explain why this hydrophobin has a stronger gushing tendency and better adsorption for hydrophobic surfaces compared to the others

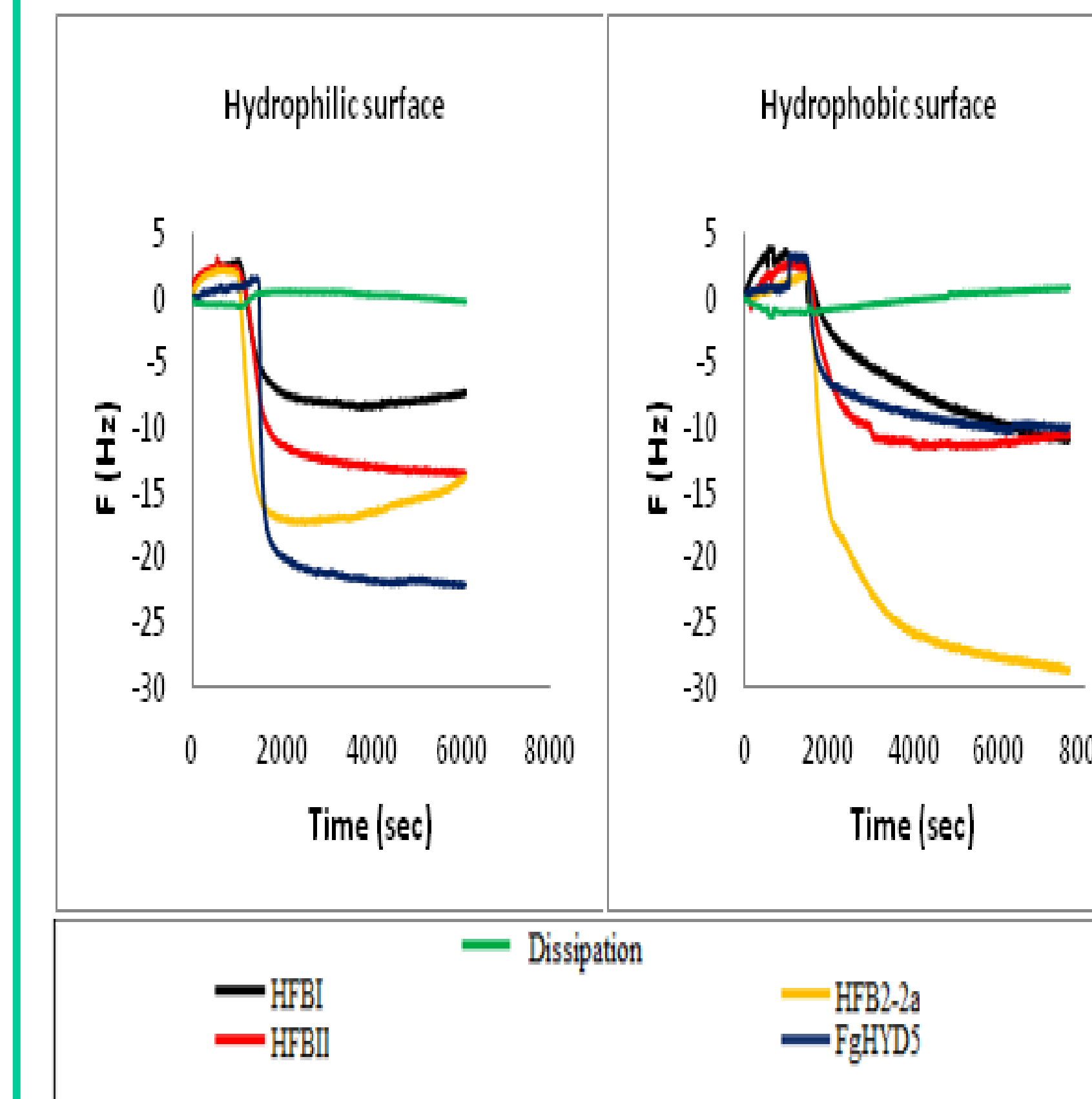


Fig 2. QCM adsorption experiments

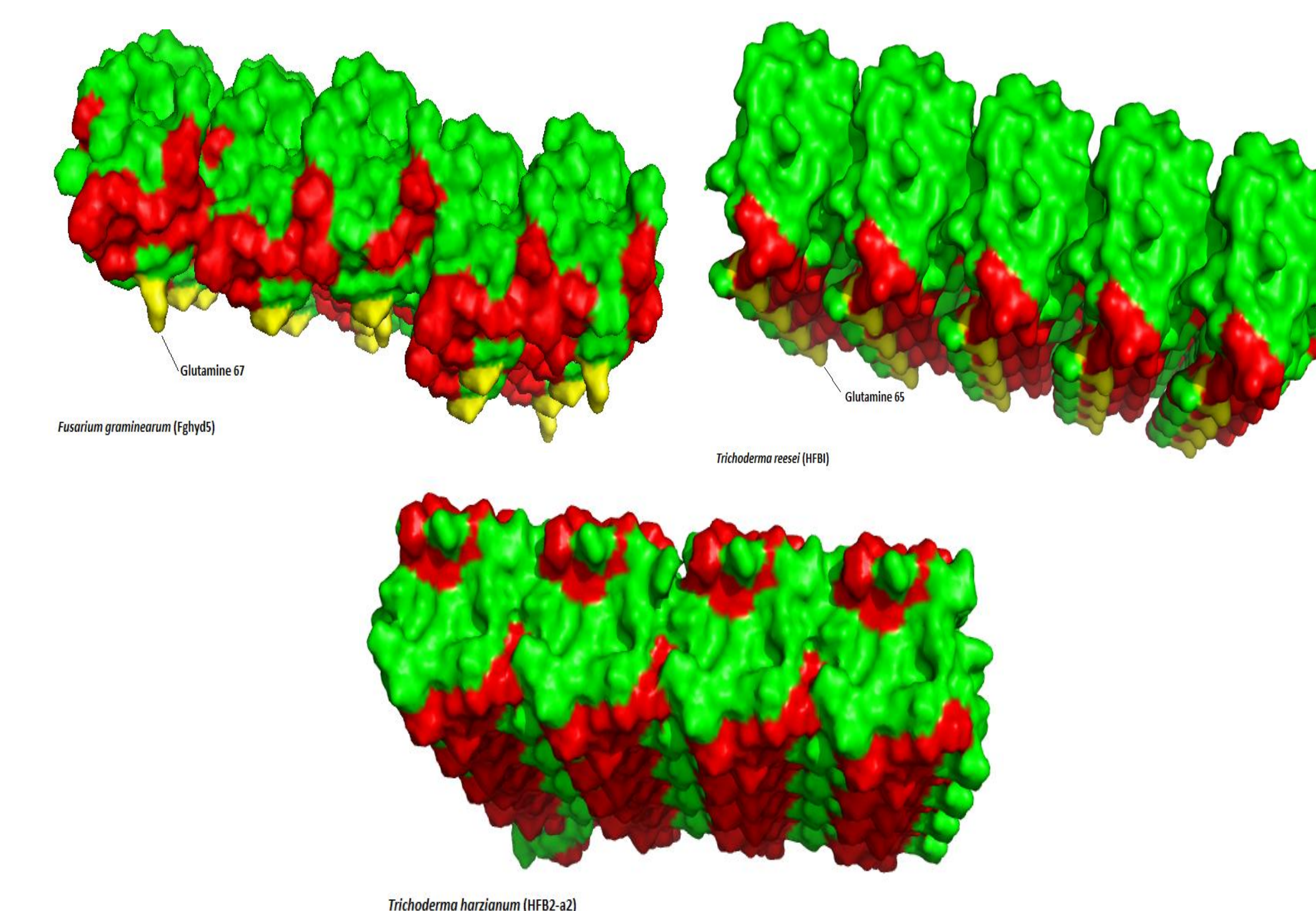


Fig 4. Hydrophobins monolayers representations hydrophobic patches colored in red

Conclusions

Self assembly mechanisms, protein-protein interactions have a definitive impact on gushing phenomenon, the presence of glutamine in the hydrophobic patch of Fghyd5, HFBI and HFBII diminish the ability to adsorption and interaction with other hydrophobic surfaces and molecules like CO₂. The uniformity and size of the hydrophobic patch is a key factor in the determination of the strenght and ability to induce gushing caused by hydrophobins.

References:

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